TRANSLATION

I, Yuko Mitsui, residing at 4-6-10, Higashikoigakubo, Kokubunji-shi, Tokyo, Japan, state:

that I know well both the Japanese and English languages,

that I translated, from Japanese into English, Japanese Patent Application No. 2000-087504, filed on March 27, 2000, and

that the attached English translation is a true and accurate translation to the best of my knowledge and belief.

Dated: January 20, 2005

Yuko Mitui

PATENT OFFICE
JAPANESE GOVERNMENT

This is to certify that the annexed is a true copy of the following application as filed with this Office.

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[Title of the Invention] ANTIGEN/ANTIBODY REACTION USING FLUORESCENT CORRELATION SPECTROSCOPY

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[List of Items Submitted]

[Name of Item]	Specification	1
[Name of Item]	Drawings	1
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[Document] SPECIFICATION

[Title of the Invention] ANTIGEN/ANTIBODY REACTION USING FLUORESCENT CORRELATION SPECTROSCOPY

[What is claimed is:]

[Claim 1] A method for detecting an antigen, comprising:

(1) placing a test sample and a plurality of antibodies respectively having a specificity to a plurality of antigens contained in the test sample and labeled with a fluorescent substance, in a same vessel;

(2) measuring fluorescent intensity at a plurality of time points in the course of the reaction;

(3) converting the fluorescent intensity obtained in the measurement using an autocorrelation function, thereby determining a binding state of a fluorescent marker antibody; and

(4) determining the presence/absence of an antigen based on the binding state of the fluorescent marker antibody.

[Claim 2] A method for detecting a surface antigen of blood cells, comprising:

(1) placing a test sample and a plurality of antibodies respectively having a specificity to a plurality of surface antigens of blood cells contained in the test sample and labeled with a fluorescent substance, in a same vessel;

(2) measuring fluorescent intensity at a plurality of time points in the course of the reaction;

(3) converting the fluorescent intensity obtained in the measurement using an autocorrelation function, thereby determining a binding state of a fluorescent marker antibody;

and

(4) determining the presence/absence of an antigen based on the binding state of the fluorescent marker antibody.

[Claim 3] A method for detecting an antibody, comprising:

(1) placing a test sample, a plurality of antigens having a specificity to a plurality of antibodies contained in the test sample, and antibodies corresponding to the former and labeled with a fluorescent substance, in a same vessel;

(2) measuring fluorescent intensity at a plurality of time points in the course of the reaction;

(3) converting the fluorescent intensity obtained in the measurement using an autocorrelation function, thereby determining a binding state of a fluorescent marker antibody; and

(4) determining the presence/absence of an antibody based on the binding state of the fluorescent marker antibody.

[Claim 4] A blood transfusion method according to any one of claims 1 to 3, characterized by comprising:

measuring a reaction between a test sample and reagent in the reaction vessel, using the Brownian movement of fluorescent molecules that they carry, according to a fluorescent correlation spectroscopy that measures the size, number, or physical quantity such as shape of molecules.

[Detailed Description of the Invention]

[0001]

[Technical Field of the Invention]

The present invention related to a method for detecting an antigen or antibody, and more specifically, to a method for

detecting antigen/antibody reaction by measuring, with time, fluctuation of molecules in the antibodies or the like with a detection system, thereby determining the type of antigen or antibody present in a reaction system. In particular, the present invention is useful in a method of blood transfusion test for testing items, such as blood types of blood cells, required to be examined with respect to the blood supply or a subject to receive blood transfusion.

[0002]

[Prior Art]

Most of the blood transfusion tests currently used in clinical treatments are based on detecting coagulation images. For example, a PK7200 manufactured by Olympus CO., LTD. is a full-automatic blood confusion test apparatus widely used in determination tests for the blood types of blood cells, infectious diseases or the like and is featured in the bottom surface of a reaction well included in a microplate used in the test. More specifically, the reaction well bottom surface has a cone-shaped hollow on which grooves having a width of a few μm. When a test blood cell and an antisera reagent, or a test serum and a controlled blood cell are mixed and subjected to reaction, if specific reaction is caused in the reaction well after a predetermined time, the blood cell and blood serum are bound to each other by antigen/antibody reaction to form a coagulation, and the coagulation is held by the groove of the well bottom surface. As a result, the coagulation is optically detected as a uniform image that has been spread the entire bottom surface of the well. On the

other hand, if specific reaction is not caused, the coagulation is not formed. As a result, the blood cell runs out of the groove and is precipitated in the lowest portion of the well bottom surface to form a rounded and concentrated image. The PK 7200 optically detects the coagulation image (positive) and noncoagualtion image (negative) by a CCD camera to determine ABO blood type and the like. Such a detection device obtains a clear and stable reaction image to thereby performing the detection with high sensitivity. However, it takes reaction time of 20 to 80 minutes to form the coagulation pattern. Further, 50 μL of reaction liquid is required. As a result, cost of the reagent to be used in a reaction becomes high. In the above circumstance, reduction in time and cost has been demanded.

[0003]

A micro typing system ABD card (hereinafter referred to as MTS) manufactured by Olympus CO., LTD. is a blood type determination method using a gel column centrifugal method, and related antibody reagents, centrifuge, temperature control device, pipetting device, readout device and the like are available. The MYS uses coagulation reaction as an index. Red blood cells or a mixture of red blood cells and blood serum (or blood plasma) is injected into a gel column filled into a particular kind of microtube of a card type. The tube is then subjected to centrifugation to conduct the determination of presence or absence of the coagulation reaction at high speed and to make the result visual. In the case of using the MTS, if the reaction is negative, the blood cells are not

coagulated and precipitated in the bottom of the microtube. If the reaction is positive, the blood cells are coagulated and captured between the upper portion and intermediate portion of the gel. That is, the determination is made based on the coagulation position of the blood cells. The method can perform semi quantative analysis and can thus determine that the reaction exhibits slightly positive. As compared to the blood transfusion test system using test tubes or microplates, this method can make determination in shorter time, more clearly, and with less test blood. Further, operation is simple and the reaction image is stable for a long time period. However, the method has not achieved speedy operation, which has been demanded in recent years. Further, the MTS requires incubation time required for forming a coagulation pattern, increasing cost of the reagent and the numbers of peripheral equipment. In view of this, the MTS has not outstripped the microplate method.

[0004]

[Objects of the Invention]

The present invention is therefore to reduce the examination time and simplify the examination step as well as to reduce the amounts of test samples and reagents.

[0005]

[Means for Achieving the Objects]

The inventors of the present invention carried out extensive studies, and as a result, found a means for solving the above problems and attaining the object.

That is, according to the present invention, there is

provided a method for detecting an antigen, comprising:

(1) placing a test sample and a plurality of antibodies respectively having a specificity to a plurality of antigens contained in the test sample and labeled with a fluorescent substance, in a same vessel;

(2) measuring fluorescent intensity at a plurality of time points in the course of the reaction;

(3) converting the fluorescent intensity obtained in the measurement using an autocorrelation function, thereby determining a binding state of a fluorescent marker antibody; and

(4) determining the presence/absence of an antigen based on the state of the fluorescent marker antibody.

[0006]

[Embodiments of the Invention]

1. Measurement principle

The present invention provides a method for determining whether or not an antigen/antibody reaction takes place. This can be performed by directly observing the movement of the labeled antibody by fluorescent correlation spectroscopy and by analyzing the state of labeled antibody.

[0007]

Thus, according to the present invention, it is possible to determine blood types of blood cells, more specifically, a grouping test and a reverse grouping test with respect to red blood cell types, and to detect irregular antibodies, and anti-blood cell specific antigens and antibodies, and an antibodies of viruses and bacteria.

[0008]

More specifically, to detect an antigen or an antibody as a detection target in a test sample, use is made of a fluorescent-substance labeled antibody or antigen which is specific to the antigen or antibody mentioned above. The antigen or antibody is first reacted with the fluorescent-substance labeled one. Subsequently, fluctuation of the fluorescence intensity of the fluorescent substance is measured with time. The obtained data is converted by a fluorescent autocorrelation function. As a result, the number and size of molecules labeled with a fluorescent substance can be determined.

[0009]

In the method according to the present invention, it is possible to measure the state of the movement of the marker molecules without the need of separating any substance present in a test sample, by an operation such as B/F separation, from the beginning to the end of the determination process. In this example, the movement of the marker molecules is determined by continuously monitoring it with time starting from the time a sample and an arbitral reagent are added to a reaction vessel and the time period of a reaction taking place in the vessel. Therefore, it is possible to determine the movement of the marker molecules changed with time by the antigen/antibody reaction. Furthermore, the determination can be accurately performed under natural conditions which vary depending upon the antigen/antibody reaction proceeding in the reaction system mentioned above.

[0010]

When the present invention is applied to the blood-type test, it is possible to capture the reaction between the antigen or antibody and labeled reagent that specifically takes place in the respective blood types at one blood cell level, measure a marker substance at a plurality of time points, and detect the positional change of the marker substance with time. Since this positional change is caused depending on the molecule size, it is possible to dynamically measure the proceeding of the specific reaction in the case where the maker reagent is bound to the target included in the test sample by quantitatively detecting the positional change.

[0011]

The method according to the present invention does not require steps other than a specific reaction, that is, a coagulation reaction step, washing step and a step of forming a centrifugal-reaction coagulation pattern which are performed in a conventional transfusion test. Furthermore, in this method, determination can be stated immediately after a sample and a reagent are mixed. Therefore, it is possible to reduce the examination time and simplify the examination step and lower occurrence of a nonspecific reaction, compared to a conventional method.

[0012]

It is possible to use a fluorescent correlation spectroscopy to be described later in order to observe the fluctuation of a fluorescent intensity.

[0013]

2. Fluorescent correlation spectroscopy

The principle and the like of a fluorescent correlation spectroscopy (hereinafter, referred to as FCS) will be described below (see "method for detecting target nucleic acid using amplification reaction of nucleic acid sequence") patent applied by Masataka Kinjyo, which includes the measurement principle of FCS, optical system and the like).

[0014]

FCS is a technique of measuring the movement of the molecules labeled with a fluorescent marker and converting the obtained data using autocorrelation function, thereby accurately measure the micro movement of the individual target molecule. As to the operation method for analyzing data obtained by measuring characteristics of a biological material by FCS, the report of Kinjo et al. can be referred to (Kinjo, M., Rigler, R., Nucleic Acids Research 23, 1795-1799, 1995).

[0015]

If FCS is used, it is possible to monitor in real time the density of the fluorescent molecules and interaction between molecules contained in the homogeneous solution through fluorescent "fluctuation" derived from Brownian movement of the average number of fluorescent molecule observed in a micro space under a microscopic view. Further, the above monitoring is achieved without a physical separation step. As described above, it is possible for the FCS to detect free movement of the molecules in the solution, so that the FCS is expected to

be applied to a wide range of research.

[0016]

3. FCS device

FIG. 1 shows a basic measurement system. An FCS device comprises an inverted fluorescent microscope 1 using a confocal optical system, a photomultiplier 2 for measuring fluorescence emitted from a sample, a data processing device 3 for receiving measurement data and calculating them by an autocorrelation function to convert them into numerical values or graphic plots, and a display unit 4 for displaying the operation results on a screen.

[0017]

A sample-containing solution 11 is easily set, as shown in FIG. 1, in such a manner that the solution 11 is dotted on a slide glass 13 placed on a sample base 12. Since a small amount of sample-containing solution is used in this device, a cover 14 is placed on the slide glass 13 in order to prevent a moisture content from vaporizing. A low light-transmissible material is preferably used as the cover 14. Air tightness and light-tightness can be simultaneously ensured by the presence of the cover. It is further preferable that a material having a light reflectivity as low as possible be used as the inner surface of the cover in order to prevent excitation light from reflecting. An objective lens 15 is arranged right under the portion of the slide glass 13 on which the sample-containing solution 11 is dotted so as to focus within the sample-containing solution 11.

[0018]

Note that the fluorescent microscope 1 may be a reflection type. In the reflection type, the sample-containing solution 11 may be dotted directly on the lower surface of the objective lens 15. In the example shown in FIG. 1, an argon (Ar) ion laser is used as a laser-generating device 16, which is a light source of the fluorescent microscope 1. Alternatively, a krypton-argon (Kr-Ar) ion laser, helium-neon (He-Ne) laser, helium-cadmium (He-Cd) laser may be used depending upon a type of a fluorescent substance. If necessary, various operations such as the loading/unloading of the slide glass 13 into/from the fluorescent microscope 1, dotting of a sample-containing solution onto the slide glass 13 and open/shut of the cover 14 may be performed automatically.

[0019]

FIG. 2 is a magnified view of a measurement portion of the fluorescent microscope 1 of FIG. 1. In FIG. 2, a micro space 20 is formed which is defined by the positions of the slide glass 13 and the objective lens 15 having a predetermined aperture (FA = 1.2 in the figure). As shown in FIG. 3, the micro space 20 is actually a focal point of laser light having a volume. The shape of the micro space 20 is nearly a cylindrical shape stretched up and down from a constricted middle portion. The field of vision 20 is restricted by the length Z on the optical axis and an average radium W based on the reference position as a focal point. In the micro space 20, fluorescence of individual fluorescent molecules can be accurately measured. This is because the volume of the micro

space 20 is reduced to the minimum sufficient to monitor the micro movement of the fluorescent molecules. With this structure, it is possible to remove the noise derived from the fluorescent molecules and present except the vicinity of the focal point of the sample-containing solution 11.

[0020]

In the foregoing, an example of the FCS device is described. However, the present invention will not be limited to the aforementioned example. Each of the contents of the embodiments of the present invention can be modified and altered.

[0021]

The detection means for measuring the movement of micro molecules preferably has a means having an optical system in order to measure the movement in a micro field of view which is set in a diffraction-limited region near a focal point. Alternatively, a measurement means preferably has a microscope to perform measurement in a micro field of view formed by the confocal optical system.

[0022]

Since the micro field of view employed in the measurement step is formed by a confocal optical system, measurement data having a deep depth-of-field can be obtained. By virtue of this, some of individual marker molecules always come into a focus in a field of view, so that an accurate portion and output data can be supplied to the measurement means. Since the micro field of view is a diffraction-limited region near a focal point, the individual marker molecules can be measured

in a high S/N ratio.

[0023]

It is preferable that the diffraction-limited region be formed by an aperture having an average diameter of 30 ± 20 μm. Further, it is preferable that the diffraction-limited region be formed by an aperture having an average diameter of 20 ± 10 μm. It is preferable that the micro field of view be nearly a cylindrical region having an average diameter of 200 ± 50 nm and an average diameter on the optical axis of 2000 ± 500 nm.

[0024]

When the micro space is nearly a cylindrical region having an average diameter of 200 ± 50 nm and an average diameter on the optical axis of 2000 ± 500 nm, as describe above, it is possible to capture a free micro movement of the marker molecules brought into within the measurement field of view.

The diffraction-limited region is formed by a pin hole of 15 ± 5 μm in average diameter. Therefore, the measurement data of a small number of marker molecules selected can be efficiently obtained.

[0025]

The output signal intensity of marker molecules (one or more molecule) is measured in a predetermined space. Furthermore, the increase or decrease of the output signal intensity thus obtained is employed as an index. Based on variation of the output signal intensity, it is possible to obtain the moving speed of the marker molecules moving in or

out of a micro measurement field of view. Therefore, the marker molecule to be labeled to a probe is preferably a marker material which can output a signal with a constant intensity at any time points. Examples of fluorescent dyes to be used as a marker include various known materials such as DAPI, FITC, rhodamine, Cy3, CY3.5, Cy5, Cy5.5, and Cy7.

[0026]

Further, an antibody reagent in a plurality of items can be determined by marking it with the different types of fluorescent substances. Furthermore, a method of changing a molecular weight of an antibody by modifying a molecule with a protein which will not affect an antigen-antibody reaction. In this case, detection is made based upon the observation which antibody reagent (more specifically, which molecular-weight) has been changed in moving speed. Multiple items can be determined by using a single fluorescent substance.

[0027]

When the positional change of a fluorescent molecule in a liquid is measured, fluorescence may be detected in the form of data by using a fluorescent measurement means such as a photomultiplier or a photodiode serving as a detection means. Furthermore, the fluorescent measurement means may have a measurement mode capable of detecting a single photon in order to measure individual fluorescent molecules.

[0028]

In this measurement step, the micro-movement of the target molecules is accurately measured by measuring the fluctuation movement of the marker molecules in a liquid.

The measurement of the fluctuation movement may be performed through the operation using the autocorrelation function. In particular, since a fluorescent substance is used as the marker molecule in the present invention, it is preferable that fluorescence correlation spectroscopy (hereinafter simply referred to as "FCS") be employed.

[0029]

While it is possible to use any measurement means as long as it can measure the change in the size of marker molecules involved with antigen/antibody reaction, a fluorescent microscope can effectively be used. The measurement step of the present invention is performed in a three-dimensional micro field of view. By virtue of this, a free micro movement of the target molecules in a sample-containing solution can be measured with a high accuracy depending upon the antigen/antibody reaction. Assuming that the detection step is performed by carrying out the measurement in a two-dimensional field of view, it is impossible to capture a three-dimensional free movement, such as the Brownian movement, of the marker molecules. As a result, the measurement accuracy becomes low. Further, the micro field of view can be obtained by such an optical design that light beams are emitted from an aperture (pin hole, optical fiber end surface, etc.) of an extremely small average diameter). Converged light of laser beams is preferable.

[0030]

The laser light, which is excitation light, is focused on only a single point of a sample solution. The fluorescent

light emitted from the point can be captured by a detection system due to the characteristics of the confocal optical system. Actually, the measurement region of a vessel is not an ideal point but a cylindrical region shown in FIG. 3. Any size (volume) of measurement region may be used. For example, the size may be about 500 nm (diameter) × 2000 nm (axial length). The volume may be in the order of femto-litle. The FCS measurement region is a solution. The fluorescent molecules present in the region move in accordance with the Brownian movement. Therefore, the number of molecules present in a predetermined measurement region is not always constant. The number of molecules fluctuates up and down from a certain value. This is called as "fluctuation in the number". Furthermore, due to the fluctuation in the number, the intensity of the measured fluorescence fluctuates. This is called "fluctuation in fluorescence intensity". By analyzing the fluctuation in fluorescence intensity, data for diffusion speed and the number of molecules can be obtained.

[0031]

It is preferable that an evaluation means comprise a memory means for storing a plurality of measurement data items in a predetermined time and an operation means for operating the stored measurement data items by an autocorrelation function. Further, it is preferable that the detection means comprise a memory means for storing measurement data items obtained in a predetermined measuring area and a calculation means for calculating the stored measurement data items separately per marker molecule by

an autocorrelation function.

[0032]

It is preferable that a data output means include a conversion means for converting the results obtained by the autocorrelation function into statistical data, which express a positional change with time with respect to a plurality of data items monitored.

[0033]

The evaluation means comprises a memory means for storing a plurality of measurement data items in a predetermined time and an operation means for operating the stored measurement data items by an autocorrelation function. Further, the detection means comprises a memory means for storing measurement data items obtained in a predetermined measuring area and a calculation means for calculating the stored measurement data items separately per marker molecule by an autocorrelation function.

[0034]

The data output means includes a conversion means for converting the results obtained by the autocorrelation function into statistical data, which express a positional change with time with respect to a plurality of data items monitored.

[0035]

The reaction vessels which can be used in the present invention include the aforementioned slide glass on which a sample can be dotted (FIG. 4), a microplate having a plurality of micro wells (FIGS. 5 and 6), and a plate having an appropriate number of micro-wells (FIGS. 7, 8 and 9). In

this case, since only a small volume is required for a reaction, an ultra micro well plate having, for example, 384 wells is applicable. If the ultra micro well plate is used, a blood test can be performed at an extremely high speed and with a high throughput.

[0036]

4. Autocorrelation function

The data measured and obtained by the above device is analyzed as explained below. A fluorescent signal is measured for about one minute. The obtained fluorescent signals are sequentially stored in a memory portion and simultaneously applied to a fluorescent autocorrelation function G(t) to evaluate them. These procedures may be previously programmed. The fluorescent autocorrelation function G(t) is performed in accordance with Equation 3 below based on an average number N of fluorescent molecules within a measurement region, translational time (τ_{mono}) and translational time (τ_{poly}) in accordance with a method of Rigler et al. (see Fluorescence Spectroscopy - new methods and applications, Springer Berlin, 13-24, In J. R. Ladowicz (Ed.), 1992). The time τ_{mono} is the translational time of free substrate molecule labeled with a marker, which serves as a nonreactive molecule including a fluorescent marker substance. The time τ_{poly} is the translational time of the labeled substrate molecule bound to a target molecule after an antigen/antibody reaction.

[0037]

[Chemical formula 1]

$$G(t) = 1 + \frac{1}{N}\left[\left\{\frac{1-y}{1+\frac{t}{T_{mono}}}\sqrt{\frac{1}{1+S^2\cdot\frac{t}{T_{mono}}}}\right\} + \left\{\frac{y}{1+\frac{t}{T_{poly}}}\sqrt{\frac{1}{1+S^2\cdot\frac{t}{T_{poly}}}}\right\}\right] \quad \ldots(1)$$

[0038]

In Equation 1, y is a ratio of a reaction component, $\tau_{mono}=Wo^2/4D_{mono}$, $\tau_{poly}=Wo^2/4D_{poly}$, S=Wo/Zo (where, Wo is a diameter of a volume element of a nearly cylindrical measurement region formed in a micro field of vision near a focal point (see FIG. 3), and 2Zo refers to the length of the volume element). D_{mono} and D_{poly} are translational diffusion coefficients of a non-binding component and a binding component, respectively.

[0039]

According to the FCS, it is possible to obtain two important parameters in the field of biochemistry, which are an average number of molecules and a translational diffusion coefficient in a detection area (10^{-15}L). In the present invention, the FCS is used as a tool for the measurement of one molecule to perform analysis related to antigen/antibody reaction.

[0040]

A preferred embodiment of the present invention below shows a method of identifying a blood type which includes the steps of detecting a time-dependence change of data before and after a specific reaction, converting the data, and detecting the size of molecules based on the diffusion speed as shown in FIGS. 10 to 13. Further, another embodiment of the present invention finds applications in a wide variety of fields including not only methods related to a blood type described later but also determination tests for infectious diseases by viruses or bacteria and determination tests by means of an antigen-antibody reaction.

[0041]

[Example 1]

FCS measurement

In the following example, FCS measurement was performed by using the FCS device shown in FIG. 1. To explain more specifically, a sample was attached in the form of a liquid drop onto a sample slide and placed on a commercially available device (ConfoCor, CarlZissJe naGmbH). The sample liquid drop was excited by a CW Ar+laser beam passing through an object lenz of 40 × magnification (C—Apochromat, NA = 1.2). The resultant emission light was measured in the form of a fluorescent signal by an avalanche diode (APD), SPCM-200-PQ (manufactured by EG & G Co., Ltd.) in a single photon counting mode. The fluorescent signal thus measured was analyzed and evaluated by a digital corelater, ALV 5000/E (ALV GmbH). The volume of the sample near a focal point region was determined

based on a diffusion coefficient of rhodamine 6G. Furthermore, the volume element was determined by using a concentration of fluorescein and a Flu-dUTP solution.

[0042]

(Example 1) Blood grouping test for determining a blood type of red blood cells

An antibody reagent labeled with fluorescence such as FITC was dispensed into a control well of a measurement vessel of a slide glass having a plurality of recesses, as shown in FIG. 8. After that, measurement is immediately carried out. The volume of the measurement vessel may be set at, for example, about 10 μl, which is sufficient as a volume for FCS measurement region. A control well and a measurement well are not necessarily set separately. The measurements before and after addition of a reagent may be regarded as a control and a test group.

[0043]

The concentration of red blood cells in a sample to be used may be low compared to that to be used in a general blood grouping test. For example, the red blood cell concentration is 10^3 - 10^4 cells/μL, more preferably, 2 to 5×10^3 cells/μL. Therefore, if the method of the present invention is applied to the blood-type test, the test can be carried out in a smaller volume of blood than a conventional test. To be more specific, the dilution factor of whole blood may be about 0.1%.

[0044]

As a reaction system, other than the reaction system as

shown in FIG. 9, a system as shown in FIG. 7 may be used. In the system of FIG. 7, an anti-A antibody and an anti-B antibody labeled with FITC are tested in separate wells, whereas an anti-A antibody labeled with FITC and an anti-B antibody labeled with Cy3 are mixed in the same well, in the system of FIG. 7. Determination is made based upon a change in mobility of a fluorescent substance used as a marker. If the mobility changes, it is determined that the antibody has reacted with blood cells (positive reaction (+)). If the mobility does not change, it is determined that the antibody has not reacted with blood (negative reaction (-)).

[0045]

[Table 1]

	Blood type of blood cells tested			
	A	B	O	AB
FITC (anti A)	+	-	-	+
Cy 3 (anti B)	-	+	-	+

[0046]

According to the reaction method, measurement can be made by only one step in which an antigen and an antibody are mixed. Therefore, incubation and centrifugal operation required for forming a coagulation pattern are not required. In addition, since the measurement region is extremely small, the amounts (volumes) of blood cells and an antibody reagent to be used in a reaction may be small. As a result, the reaction cost can be reduced. Furthermore, the reaction between a single antigen

and a single antibody can be directly captured simply based upon a change in mobility of a fluorescent molecule without using a solid surface and a coagulation pattern. Therefore, there is less chance of causing a nonspecific reaction. Hence, quantitational determination can be made by using a numerical measurement value. This is an important feature in solving a problem of a transfusion test, that is, determination of a blood subtype.

[0047]

Furthermore, as shown in FIG. 7, a plurality of items with respect to a single test sample can be determined in the same vessel. It is therefore possible to reduce the measurement time and the amounts of test samples and reagents, etc.

[0048]

Furthermore, the sample of the reaction system may be stirred by an appropriate means such as an ultrasonic wave.

[0049]

(Example 2) Reverse grouping test for determining a blood type of red blood cells

After the standard blood cells of A type and B type and a serum to be tested are mixed, the mixture is added to the well in which a standard antibody labeled with fluorescence has been dispensed. Immediately upon mixing, measurement is immediately performed. In this case, the determination is principally performed based upon a competitive reaction of an anti-blood type antibody present in a test serum and the standard antibody.

[0050]

By using different fluorescent substances in the same as in the grouping test, the standard blood cells of both A type and B type and fluorescence-labeled standard antibodies of both anti A type and anti B type are mixed in the same well per test sample and subjected to a reaction.

[0051]

Determination is made based upon a change in mobility of a fluorescent substance serving as a marker. If the mobility changes, it is determined that the antibody has reacted with blood cells (positive reaction (+)). If the mobility does not change, it is determined that the antibody has not reacted with the blood cells (negative reaction (-)).

[0052]

[Table 2]

	Blood type of blood cells tested			
	A	B	O	AB
FITC (anti A)	-	+	-	+
Cy 3 (anti B)	+	-	-	+

[0053]

(Example 3) Screening test for irregular antibody

To perform a screening test for irregular antibodies to red blood cells, a fluorescence-labeled anti human globulin serum serving as a secondary antibody is previously dispensed in a reaction well. Subsequently, the standard blood cells, that is, two or three types of O-type normal human blood cells

(containing sufficient antigens for determining clinically important antibodies) are generally mixed with the serum to be tested. Immediately upon mixing, the resultant mixture is dispensed in reaction wells and subjected to detection.

[0054]

The fluorescence-labeled serum can be bound to globulin other than irregular antibodies liberated in the test serum, changing the mobility of fluorescent substance. However, when the irregular antibody bound to the standard blood cells is present, the mobility of the fluorescent substance changes more significantly than the case mentioned above. Hence, the presence of the irregular antibody can be easily determined.

[0055]

(Example 4) Application

The present invention can be applied to the case where an antibody is identified after screening. In this case, the antibody is identified by using panel blood cells for antibody identification which are prepared by distributing clinically important antigens as panels. To explain more specifically, the antibody is identified by reacting the panel blood cells in a separate well and analyzing its reaction pattern.

[0056]

Furthermore, when the reaction using the standard blood cells is employed, if desired, the standard blood cells may be immobilized onto a reaction vessel. Alternatively, it may be possible to use a reaction vessel having a partition which prevents blood cells from moving in the micro field of view. With this structure, the background may be minimized.

[0057]

As described in the examples (1) to (3), the present invention can solve the problems of a transfusion test mentioned in the column of "Objects of the Invention".

[0058]

[Advantages of the Invention]

According to the present invention, it is possible to eliminate the B/F separation operation using centrifugal washing or the like, which has stood as an obstacle to simplification and automatization of the test method and reduction in the reaction time.

[0059]

Further, this method measures antigen/antibody reaction at a molecule level, so that it is possible to mix a plurality of normal blood cells and therefore to perform a screening test for irregular antibodies in one well as in the case of the grouping test and reverse grouping test.

[0060]

In the present invention, the region in which a glowing signal is to be detected is a micro space having a size in which the blood cell having a size of a few μ can be individually measured. Thus, amounts of the test sample and reagent required for the measurement are extremely small. Therefore, the test having a plurality of items to be determined, such as a blood type, can be performed with small amounts of test sample and reagent. Further, all blood types can be contained in a small vessel. This reduces the amounts of test sample and reagent, as well as performs the test at

high speed to shorten the test time.

[0061]

In the conventional method for determining polymorphism such as a blood type, respective polymorphisms must be subjected to reaction in different vessels prepared in accordance with the number of the polymorphisms. However, in the present invention, it is possible to easily detect the positional change takes place in which of the fluorescent substances by marking reagents having different specificities with different fluorescent substances. Therefore, a plurality of reagents having specificities to different polymorphism sites can be mixed in the same vessel, so that a plurality of tests can simultaneously be determined. As a result, not only the amount of test sample but also the number of reaction vessels can be reduced. In addition, a polymorphism gene test can be easily performed.

[0062]

Moreover, the polymorphism gene test can be performed at least with a mixture of a test sample and reagent, so that the entire system can be automated.

[0063]

In the conventional methods, when reagents having different specificities are subjected to reaction in different vessels prepared in accordance with the specificities thereof and polymorphisms, a 96 well plate type or a vessel having a shape and size corresponding the 96 well plate must be used. In theses conventional methods, large amounts of blood sample serving as a test sample and reagent are required. In addition,

items that can be measured at a time are limited. However, according to the method of the present invention, it is possible to use not only a well but also to employ a method of dotting a slid glass with a sample. Therefore, it is possible to measure a plurality of samples with one slide glass. Further, a plurality of items can be measured with one slide glass.

[Brief Description of the Drawings]

[FIG. 1]

A view showing an example of a FCS device which can be used in a detection method according to the present invention.

[FIG. 2]

An enlarged view of a measurement portion of a fluorescent microscope included in the FCS device shown in FIG. 1.

[FIG. 3]

A view showing a micro field of view.

[FIG. 4]

A cross-sectional view showing a slide glass on which a sample is dotted.

[FIG. 5]

A view showing a microplate.

[FIG. 6]

Across-sectional view showing a microplate.

[FIG. 7]

A view showing a microplate.

[FIG. 8]

A view showing a microplate.

[FIG. 9]

A view showing a microplate.

[FIG. 10]

A view showing the movement of relatively small molecules in a micro field of view.

[FIG. 11]

A graph showing fluctuation of relatively small molecules with time.

[FIG. 12]

A view showing the movement of relatively large molecules in a micro field of view.

[FIG. 13]

A graph showing fluctuation of relatively large molecules with time.

[Explanation of Reference Symbols]

1 ... Fluorescent microscope,

2 ... Photomultiplier,

3 ... Data processing device,

4 ... Display unit,

11 ... Sample-containing solution,

12 ... Sample base,

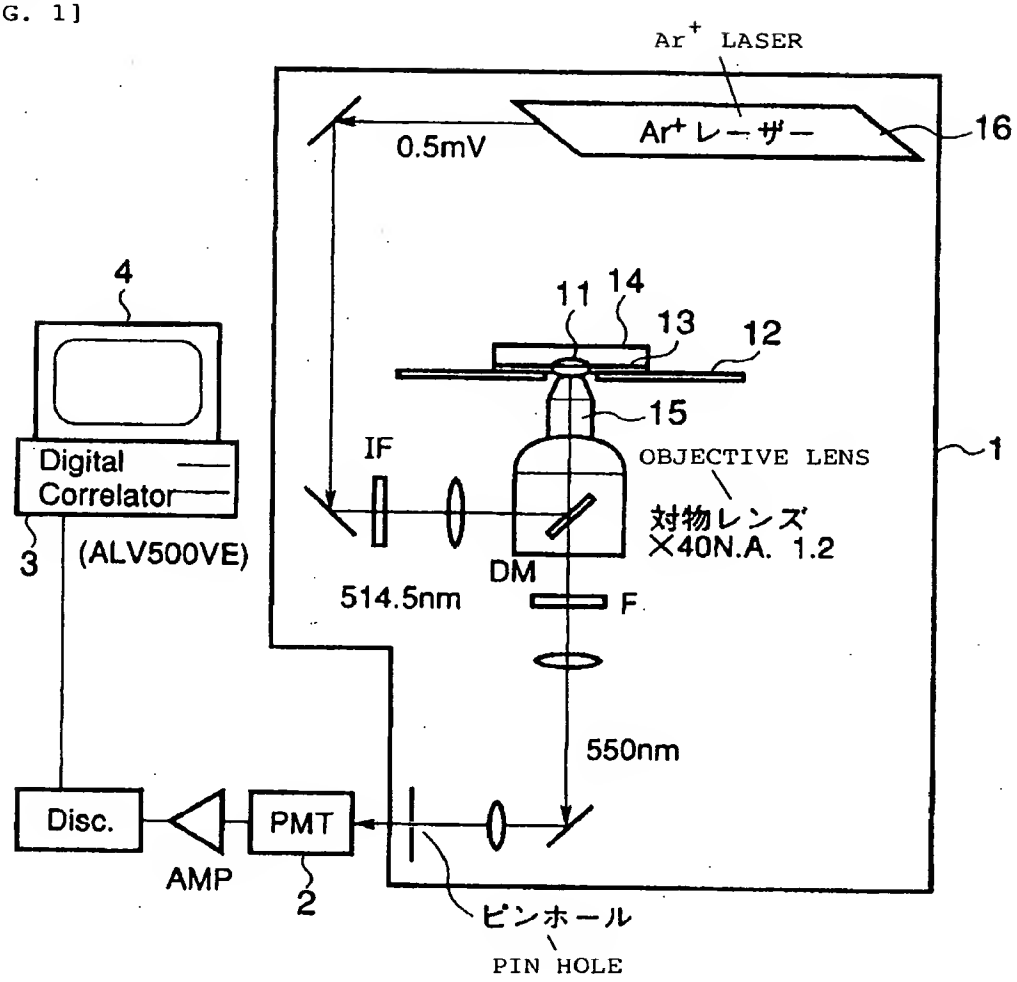
13 ... Slide glass,

14 ... Cover,

15 ... Objective lens,

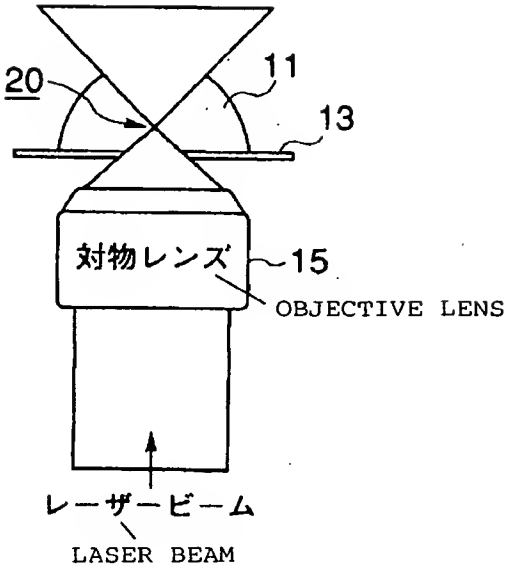
16 ... Laser-generating device.

【書類名】　図面
[NAME OF DOCUMENT]　DRAWINGS
【図１】
[FIG. 1]



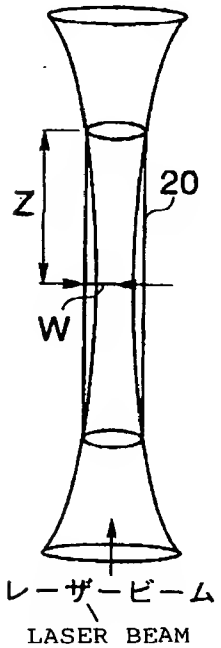
【図２】

[FIG. 2]



【図３】

[FIG. 3]



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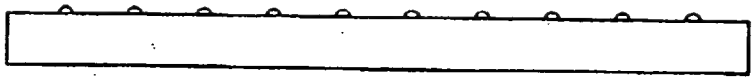
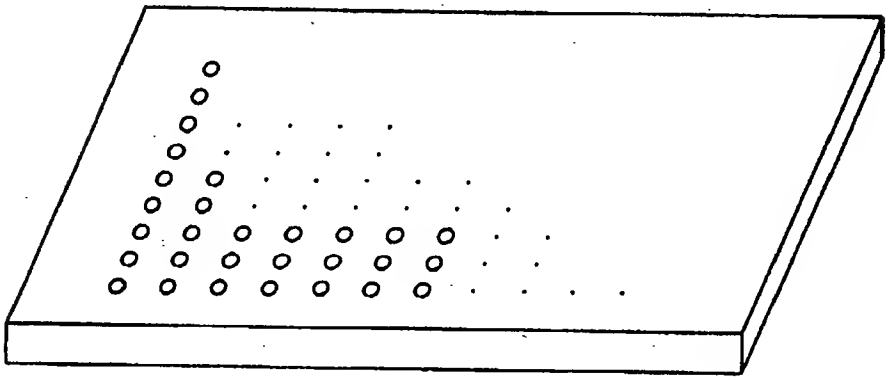
[FIG. 4]



【図５】

[FIG. 5]

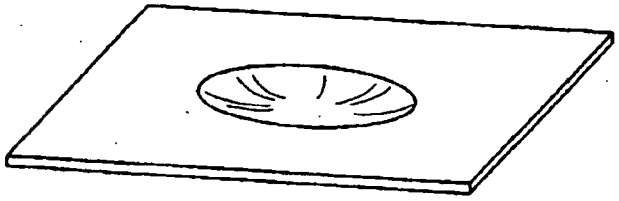


【図６】

[FIG. 6]

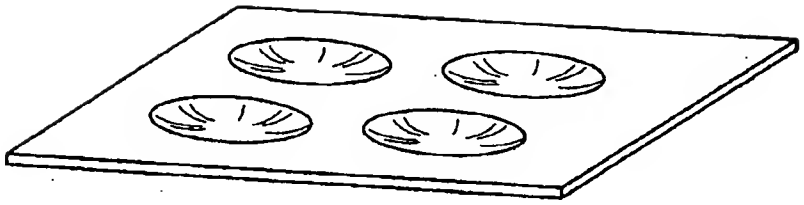
【図７】

[FIG. 7]



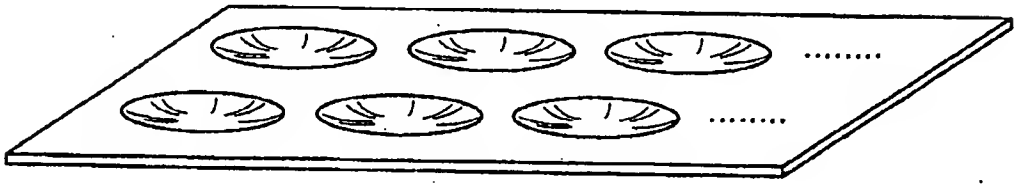
【図８】

[FIG. 8]



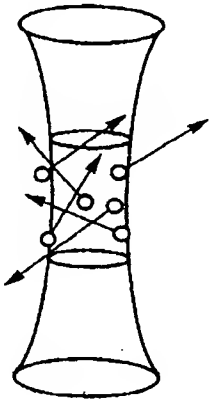
【図９】

[FIG. 9]



【図１０】

[FIG. 10]



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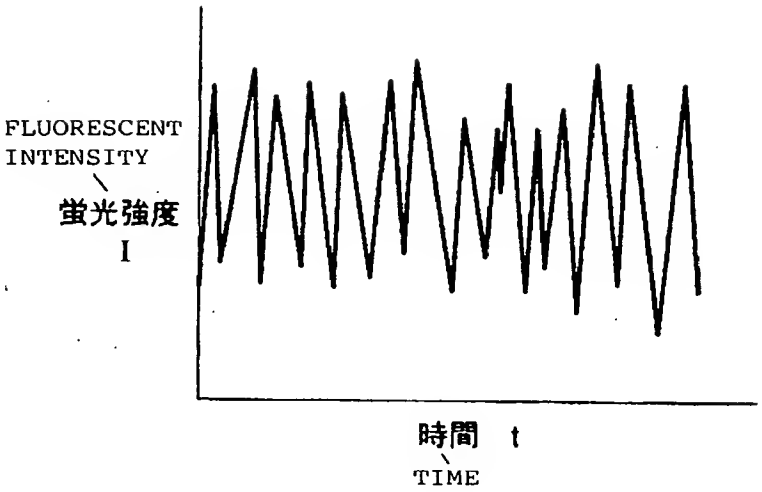
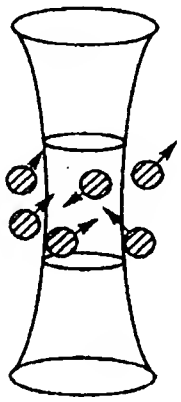
[FIG. 11]



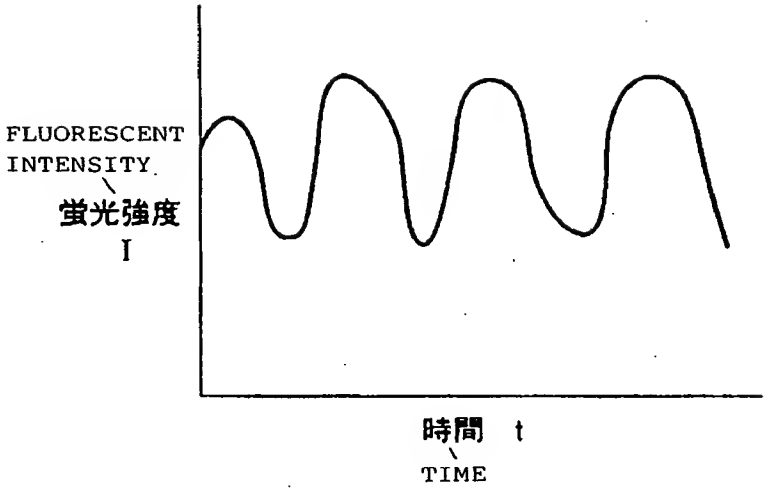
【図１２】

[FIG. 12]



【図１３】

[FIG. 13]



[Document] ABSTRACT

[Abstract]

[Objects] The present invention is therefore to reduce the examination time and simplify the examination step as well as to reduce the amounts of test samples and reagents.

[Means for Achieving the Objects] A method for detecting an antigen according to the present invention comprises:

(1) placing a test sample and a plurality of antibodies respectively having a specificity to a plurality of antigens contained in the test sample and labeled with a fluorescent substance, in a same vessel;

(2) measuring fluorescent intensity at a plurality of time points in the course of the reaction;

(3) converting the fluorescent intensity obtained in the measurement using an autocorrelation function, thereby determining a binding state of a fluorescent marker antibody; and

(4) determining the presence/absence of an antigen based on the binding state of the fluorescent marker antibody.

[Elected Figure] NONE

APPLICANT'S PAST DATA

Identification Number [000000376]

1. Date of Change August 20, 1990

[Reason for Change] New Registration

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